Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the developing Chicken Embryo-**Propylene Glycol** No Date

K25

FDA

Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo
PROPYLENE GLYCOL

Protocol:

Propylene glycol was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by the two routes at two stages of embryonic development: via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (1, 2).

Groups of 10 or more eggs were treated under these four conditions at several dose levels until a total of ninety to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. All hatched chicks and non-viable embryos were examined carefully for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively (the milligrams per kilogram figure is based on an average egg weight of fifty grams). Column 3 is the total

number of eggs treated. Column 4 is the percent mortality i.e. total non-viable divided by total treated eggs. Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders. Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent treated eggs and the untreated controls.

The mortality data in Column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (3). The results obtained are indicated at the bottom of each table.

The data of Columns 4, 5, and 6 have been analyzed using the Chi Square Test for significant differences from the control background. Each dose level is compared to the control value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

At hatchings, 3 chicks were removed at random from each level including control for skeletal clearing, weighing and fixing of bursa, spleen, liver and kidney. Tissues were processed, blocked in paraffin, sectioned, affixed to slides, and stained. Later these sections were examined for internal damage to the tissues.

Discussion:

Propylene glycol was tested at dose levels between 300 and 1000 mg/kg in two stages of embryonic development, by both air cell and yolk routes. The estimated LD-50 values for all four treatments of the test are as follows:'

Treatment	LD-50 Level
Air cell treatment 0 hours	3923.73 mg/kg (196.19 mg/egg)
Air cell treatment 96 hours	2247.69 mg/kg (112.4 mg/egg)
Yolk treatment 0 hours	1325.58 mg/kg (66.28 mg/egg)
Yolk treatment 96 hours	1606.37 mg/kg (80.32 mg/egg)

Significantly different percent mortality rates were observed mainly when propylene glycol was given via yolk at the dose level of 800 mg/kg or above. In air cell treatment, only 1000 mg/kg dose level at 96 hours of embryonic development produced a death rate which was significantly different from solvent. The LD-50 values for all the treatments were much higher than the highest dose level used in our tests.

In general, propylene glycol was neither toxic nor teratogenic under the test conditions employed between the dose ranges 300 and 1000 mg/kg.

References:

1. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

2. Verrett, M. J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1003-1006.

3. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendic I.

PROPYLENE GLYCOL
AIR CELL 0 HOURS

DOSE		Number of	Percent	Percent Abnormal	
mg/egg	mg/kg	Eggs	Mortality *	Total	Structural
50.0	1000.0	100	21.0	0.0	0.0
40.0	800.0	100	18.0	0.0	0.0
30.0	600.00	100	18.0	0.0	0.0
22.5	450.00	100	11.0	0.0	0.0
15.0	300.00	100	14.0	0.0	0.0
Water		100	14.0	0.0	0.0
Pierced Control		100	16.0	0.0	0.0

*Slope is Negative

PROPYLENE GLYCOL
AIR CELL 96 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent Mortality*	Percent Abnormal Total	Percent Abnormal Structural
50.0	1000.0	100	27.0*	0.0	0.0
40.0	800.0	100	25.0	0.0	0.0
30.0	600.0	100	20.0	0.0	0.0
22.5	450.0	100	11.0	0.0	0.0
15.0	300.0	100	14.0	0.0	0.0
Water		100	14.0	0.0	0.0
Pierced Control		100	17.0	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

PROPYLENE GLYCOL
YOLK AT 0 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
50.0	1000.0	100	38.0*	0.0	0.0
40.0	800.0	100	29.0*	0.0	0.0
30.0	600.0	100	19.0	0.0	0.0
22.5	450.0	100	13.0	0.0	0.0
15.0	300.0	100	10.0	0.0	0.0
Water		100	12.0	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

PROPYLENE GLYCOL
YOLK 96 HOURS

DOSE		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
50.0	1000.0	99	31.31 *	0.0	0.0
40.0	800.0	100	33.00 *	0.0	0.0
30.0	600.0	100	19.00	0.0	0.0
22.5	450.0	100	14.00	0.0	0.0
15.0	300.0	100	12.00	0.0	0.0
Water		100	13.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$